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Leukocyte lipid bodies - biogenesis and functions in inflammation

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Abstract

Lipid body accumulation within leukocytes is a common feature in both clinical and experimental infectious, neoplastic and other inflammatory conditions. Here, we will review the contemporary evidence related to the biogenesis and structure of leukocyte lipid bodies (also known as lipid droplets) as inflammatory organelles. Studies of leukocyte lipid bodies are providing functional, ultrastructural and protein compositional evidences that lipid bodies are not solely storage depots of neutral lipid. Over the past years substantial progresses have been made to demonstrate that lipid body biogenesis is a highly regulated process, that culminate in the compartmentalization of a specific set of proteins and lipids, that place leukocyte lipid bodies as inducible cytoplasmic organelles with roles in cell signaling and activation, regulation of lipid metabolism, membrane trafficking and control of the synthesis and secretion of inflammatory mediators. Pertinent to the roles of lipid bodies in inflammation and cell signaling, enzymes involved in eicosanoid synthesis are localized at lipid bodies and lipid bodies are sites for eicosanoid generation. Collectively, lipid bodies in leukocytes are emerging as critical regulators of different inflammatory diseases, key markers of leukocyte activation and attractive targets for novel anti-inflammatory therapies.

Keywords

Lipid droplets; inflammation; foam cell; eicosanoids; leukocytes; eosinophils; neutrophils

1.0 Introduction

Lipid bodies, also named lipid droplets or adiposomes, are lipid-rich organelles present in virtually all organisms, including plants, yeast, prokaryotes and both non-mammalian and mammalian animal cells. Although resting mammalian leukocytes contain few lipid bodies, lipid bodies characteristically increase in numbers and prominence in cells associated with diverse inflammatory responses, including leukocytes (e.g., macrophages, neutrophils, and eosinophils) and other cell types (e.g., endothelial cells). Lipid body accumulation within leukocytes is observed in both clinical and experimental infectious, neoplastic and other inflammatory conditions, including in atherosclerosis [1,2], bacterial sepsis [3,4], acute respiratory distress syndrome [5,6], allergic lung inflammation [7–9], arthritis [10–13], and in

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mycobacterial infections [14–16]. Lipid bodies are bound not by a classic bilayer membrane but rather by an outer monolayer of phospholipids, which at least in some cells may have a unique fatty acid composition [17,18]. The internal core of lipid bodies is rich in neutral lipids; and it is likely that more complex membranous domains, often obscured by overlying neutral lipids, are present within lipid bodies. Indeed, studies of leukocyte lipid bodies are providing functional, ultrastructural and protein compositional evidences that lipid bodies are not solely “bags” of neutral lipid. The presence of membranous domains within leukocyte lipid bodies is also consonant with findings pertinent to lipid bodies in adipocytes, steroidogenic cells and other cells, as we consider below. Although in the past the presence of lipid bodies in cells has been largely associated with neutral lipid storage and transport, it has become clear that lipid bodies are highly regulated, dynamic and functionally active organelles.

Lipid bodies in leukocytes and other cells compartmentalize a diverse set of proteins (Figure 1). The major structural proteins present at the surface of lipid bodies are the proteins from the PAT family [19], including perilipin [20], adipose differentiation related protein (ADRP) [21,22], and TIP 47 (tail-interacting protein of 47 kDa) [23]. These proteins have been implicated in lipid body assembly and biogenesis [21,22,24,25]. By using techniques of protein identification in subcellular lipid body-enriched fractionation combined with immunodetection of proteins by EM or light microscopy it has been shown that lipid bodies compartmentalize enzymes involved in the biosynthesis, transport and catabolism of lipids [1] [26–31], caveolin, proteins of Rab family and proteins involved in vesicular transport including VAT-1, SNAP and VAMP [29–37], eicosanoid-forming enzymes [3,38–43], protein kinases as phosphatidylinositol 3 kinase (PI3 kinase), mitogen-activated protein (MAP) kinases, and protein kinase C (PKC) [26,44,45]. The regulated formation of lipid bodies, their protein and lipid content, and their association with other intracellular organelles have established leukocyte lipid bodies as specialized, inducible cytoplasmic domains that function as organelles with roles in cell signaling and activation, regulation of lipid metabolism, membrane trafficking and control of the synthesis and secretion of inflammatory mediators. Here, we will review the contemporary evidence related to the biogenesis and structure of leukocyte lipid bodies as inflammatory organelles. Collectively, lipid bodies in leukocytes are critical regulators of different inflammatory diseases, key markers of leukocyte activation and attractive targets for novel anti-inflammatory therapies.

2.0 Leukocyte lipid body biogenesis

Different from neutral lipid storing cells, leukocytes contain few lipid bodies under resting conditions (e.g., human blood neutrophils and eosinophils contain ~ 1 and ~ 5 lipid bodies/cell [46,47]). Of note, lipid bodies in leukocytes have often not been recognized since lipid bodies are dissolved by common alcohol-based hematologic stains. However, rapid and well-regulated lipid body biogenesis is triggered upon leukocyte activation by different stimuli and pathological conditions including obesity-induced inflammation, ox-LDL- and LPS-induced inflammation or bacterial infection. Thus increased numbers of cytoplasmic lipid bodies within leukocytes are often associated with infectious, atherosclerotic and other inflammatory conditions.

Hypothesized mechanisms for the biogenesis of lipid bodies in varied cells types recognize that lipid bodies are intimately related with and likely derived from the endoplasmic reticulum (ER)(Figure 2). The proposed models of the biogenesis of lipid bodies involve ER transfer of lipids and proteins; however the precise mechanisms involved remain to be ascertained. Three main models have been proposed to explain the formation of lipid bodies and their interaction with the ER. One of the first models proposed was a “budding model” where enzymes involved in lipid metabolism accumulate in specific domains of the ER, thus favoring neutral lipid synthesis in these regions forming a hydrophobic neutral lipid mass between the two leaflets

of the ER bilayer (Figure 2 A). After reaching a certain size, nascent lipid bodies loaded with proteins lacking trans-membrane spanning domains bud off the ER into the cytoplasm that ends up surrounded by a half-unit membrane of phospholipids directly derived from the cytoplasmic leaflet of the ER [48–51].

An alternative second model has been proposed. Based on freeze-fracture immunogold electron microscopy observations, Robenek and colleagues have recently suggested that formation of lipid bodies occurs preferentially alongside, not within, the ER; and an “egg cup model” of lipid body formation was proposed (Figure 2 B). According to this model, lipid bodies would form within ER cups particularly enriched in ADRP clusters in the cytoplasmic leaflet of ER, with ADRP clusters functioning to transfer lipids from ER to nascent lipid bodies [52].

Based both on studies of leukocyte lipid bodies and on findings related to lipid bodies in other cell types, we have proposed a novel “enfolding model” of lipid body biogenesis that accommodates membrane-associated and transmembrane spanning proteins within lipid body cores by incorporation of multiple loops of ER membranous domains [31] (Figure 2 C).

Just as PAT proteins can localize at the peripheral delimiting membrane of lipid bodies, an initially attractive hypothetical mechanism for cytosolic proteins (e.g., 5-lipoxygenase (5-LO), cytosolic phospholipase A₂ (cPLA₂) involved in eicosanoid metabolism to function at lipid bodies was to have these membrane-active proteins “translocate” to the peripheral membrane of lipid bodies. Of note, however, immunogold electron microscopic localization of 5-LO revealed 5-LO to be distributed throughout lipid bodies, which were shown to have a “honeycomb” internal structure [39], never suggesting a “translocated” membrane association solely at the peripheral delimiting monolayer membrane of lipid bodies. The cyclooxygenase (COX, prostaglandin H synthase) enzymes are integral membrane proteins [53] and have been localized at lipid bodies in different cells and by the use of different techniques including EM immunogold, immunolocalization in intact cells and western blotting from subcellular fractions [3,38–40,54,55] [41–43]. Similar to the described for 5-LO, EM immunogold analysis of COX enzymes localized these proteins throughout lipid bodies in diverse cells and not restricted to the periphery of the lipid body. Analogously, the transmembrane spanning enzyme, leukotriene C₄ (LTC₄) synthase, was localized at lipid bodies [39]. As described in detailed below, eicosanoid-forming enzymes localized at lipid bodies retain their functional enzymatic capacity since newly generated arachidonate-derived eicosanoids are formed at lipid bodies in inflammatory cells [8,15,56–58]. Collectively, these findings indicated that since membrane-associated enzymes localized and functioned at leukocyte lipid bodies there would likely be internal membranes present within these lipid bodies.

Proteomic studies of lipid bodies isolated from the U937 macrophage cell line identified diverse proteins, about half of which had predicted membrane insertions [31], combined with ultrastructural observations of membranous structures within lipid bodies of U937 macrophage cell line and human eosinophils, strongly suggested the presence of internal membranes within leukocyte lipid bodies. Accordingly, lamellar concentrically arranged membranes have been previously described to occur on the margins of lipid droplets that penetrated the matrix of the droplets in acetylated LDL-stimulated macrophages [1]. The presence of membranotubular structures have also been recognized in adipocyte lipid bodies recently by embedment-free electron microscopy [59]. The existence of membranous structures within lipid bodies of non-leukocytic cells could also explain how stanniocalcin and its membrane receptor are present at lipid bodies of ovarian steroidogenic cells and adipocytes [60]. Membranes within lipid bodies would account for the freeze-fracture immunogold electron microscopic findings that revealed caveolin-1, an integral membrane protein, as well as TIP47 and ADRP were localized to freeze-fractured lamellae not only at the periphery of lipid bodies but also pervading lipid body cores [61].

Although stores of neutral lipids that accumulate at lipid bodies can obscure the internal ER-derived components of lipid bodies, ribosomal structures and ribosomal associated proteins have been described in leukocyte lipid bodies [31] [62,63]. Moreover, protein analyses of *Drosophila*, yeast and hepatoma lipid bodies also identified ribosomal and RNA-interacting proteins [64–68]. Future studies will be necessary to confirm and characterize the presence of membranous structures in the lipid body core.

The triggering process and detailed molecular mechanisms involved in lipid body biogenesis have been intensely investigated, demonstrating that leukocyte lipid body biogenesis is a highly regulated phenomena that has been characterized as a cell and stimuli dependent event [69] (Table 1).

Upon stimulation the genesis of new cytoplasmic lipid bodies can be induced within neutrophils, eosinophils, and monocytes/macrophages, as well as in other inflammation-related cell types including endothelial and epithelial cells. Studies investigating the mechanisms of lipid body formation in leukocytes, using physiological amounts of fatty acids or other relevant inflammatory stimuli, demonstrated that a complex biogenic process rather than unregulated lipid incorporation takes place in leukocytes to form new lipid bodies. Among the observation that indicate the existence of regulated production of lipid bodies in leukocyte are: (i) while *cis*-unsaturated fatty acids are potent inducers of lipid bodies, saturated fatty acids do not elicit lipid body assembly [70,71]; (ii) non-esterifiable *cis*-fatty acids such as the arachidonate analog arachidonyl trifluoromethyl ketone are able to induce formation of new lipid bodies [72]; and (iii) stimulation with cytokines/chemokines and hormones induces receptor-mediated lipid body biogenesis not only *in vivo* but even *in vitro* in the absence of exogenous lipids (Table 1).

Attempts to characterize the signaling pathways committed to lipid body biogenesis in leukocytes revealed that different pathways in a stimulus-dependent fashion are activated to trigger leukocyte lipid body biogenesis. For instance, platelet-activating factor (PAF) and PAF-like molecules, but not lyso-PAF, acting via its G-protein-linked receptor induces lipid body formation via downstream signaling that requires 5-LO, PKC and phospholipase C (PLC) activation [39,40,70,73], in a mechanism that may involve an autocrine loop on CCL2 generation (Figure 3). Prostaglandin (PG)₂ – another potent leukocyte chemoattractant acting through a G-protein-linked receptor – also directly induces biogenesis of lipid bodies in eosinophils, but not in macrophages, even though both cell types express functional PGD₂ receptors demonstrating stimulus/signaling specificity according to the cell type [9]. Of note, other G-protein-coupled receptor agonists, including IL-8, C5a and LTB₄, did not induce leukocyte lipid body formation demonstrating the requirement of specific intracellular signaling mechanisms in the process of lipid body biogenesis [70].

Leukocytes incubated with cytokines and chemokines even in the absence of exogenous lipids rapidly form new cytoplasmic lipid bodies by receptor-mediated processes [40,56,58,74,75]. In human eosinophils, IL-5 alone or combined with GM-CSF, as well as immobilized IgG lead to significant increases in lipid body numbers [40,75]. In addition, RANTES (CCL5) and eotaxin (CCL11), eotaxin 2 (CCL24) and eotaxin 3 (CCL26) acting via their CCR3 receptors, initiate intracellular signaling in eosinophils and basophils, but not neutrophils, to rapidly form lipid bodies [8,56,76]. CCR3-driven lipid body biogenesis, in contrast to PAF's effect, was mediated by activation of MAP kinases, PI3K and tyrosine kinases, but not PKC or PLC [56]. Likewise, *in vivo* administration of RANTES, eotaxin or PGD₂ also induced significant influx of eosinophils loaded with lipid bodies through their specific receptors [8,9]. Eosinophils attracted to the site of allergic inflammatory reaction exhibited increased numbers of lipid bodies [8,9]. Allergic inflammation induces *in vivo* biogenesis of lipid bodies within recruited eosinophils in a selective manner, since it failed to activate resident mononuclear cells to form

lipid bodies. Neutralizing antibodies to eotaxin, RANTES or the eotaxin and RANTES receptor, CCR3, as well as a specific inhibitor of PGD₂ synthesis inhibited lipid body formation within recruited eosinophils during experimental allergic inflammatory reactions [8,9]. Therefore, allergic inflammation triggers *in vivo* formation of new lipid bodies within infiltrating eosinophils, a phenomenon largely mediated by a cross-talk of eotaxin/RANTES acting via CCR3 receptors and PGD₂ acting via a receptor that has yet to be identified. A role for eotaxin/CCR3 in lipid body biogenesis was also demonstrated to occur in infection-driven lipid body formation in eosinophils, but not in macrophages; a mechanism that was largely dependent on TLR2-dependent macrophage-derived eotaxin-mediated CCR3 activation [77].

Accumulation of lipid body enriched macrophage foam cells in atherosclerotic blood vessel intima is a critical component of atherogenesis. The formation of foam cells involves complex and multi-step mechanisms that depend on different signaling pathways regulating lipid influx, metabolism, storage and mobilization [2,78,79]. Uncontrolled modified LDL uptake by macrophages through scavenger receptors causes triglyceride and cholesterol loading, followed by cholesterol esterification mediated by acyl coenzyme A:acylcholesterol transferase and storage of cholesteryl esters (CEs) in cytoplasmic lipid bodies [2,78,79]. Different modifications of LDL, including enzymatic modification (E-LDL), acetylation (Ac-LDL), oxidation (Ox-LDL), and glycation (AGE-LDL) have been associated to foam cell formation in atherosclerosis [2,79–82]. Recognition and activation of scavenger receptors, mostly CD36, by modified LDL play major roles in lipid accumulation in macrophages [83–87]. In addition, different lipid-derived molecules generated in the process of LDL oxidation are involved in lipid body formation including PAF-like molecules [73,88], sterol ester [89], oxysterols [90], 1-palmitoyl-2-(5'-oxovaleroyl)-sn-glycero-3-phosphocholine [91], azelaoyl-phosphatidylcholine [73].

Adipocytokines including leptin and resistin were also shown to modulate lipid body formation in macrophages and may participate in the mechanisms of foam cell formation [92–94]. In addition, current studies have demonstrated that monocyte chemoattractant protein (MCP-1/CCL2), a key endogenous mediator involved in the pathogenesis of macrophage-driven inflammation such as atherosclerosis and sepsis, is centrally involved in the regulation of macrophage lipid body biogenesis in oxidized LDL- and LPS-induced inflammation as well as in experimental sepsis [58,88], Silva, submitted). MCP-1-driven lipid body accumulation is a highly regulated phenomenon, requisitely dependent of MCP-1 receptor, CCR2, and downstream signaling through MAP- and PI3-kinases [58]. Moreover, MCP-1-elicited lipid body assembly and protein compartmentalization was demonstrated to depend on a functional microtubule network. Accordingly, lipid bodies are enmeshed in a cytoskeleton network in several cell types [31,95–97], and lipid body-cytoskeleton interaction were shown also to have roles in lipid body motility [36], rapid relocation upon cell activation with chemotactic agents [56], and lipid body fusion and growth [98] (Figure 3).

Whether inflammation-driven lipid body formation depends on either a direct effect of endogenous mediators present at the site of inflammatory reaction or is triggered by cellular migration and/or phagocytosis dependent mechanisms at the inflammatory site has been investigated. Attempts to answer the role of cell migration – a multi-step process that involves rolling, adhesion, transmigration and chemotaxis – to lipid body biogenesis indicate that migration may modulate, but is not requisitely involved in leukocyte lipid body formation during inflammation. Increased lipid body biogenesis may be dissociated from cell recruitment as (i) resident macrophages have significantly increased lipid body numbers within 6 hours of LPS stimulation when there is no concurrent increase in the number of macrophages at the inflammatory site [3]; (ii) while *in vivo* infection with *Mycobacterium bovis* BCG induced both migration and lipid body formation within recruited leukocytes, infection with non-pathogenic *Mycobacterium smegmatis* although inducing an intense leukocyte recruitment, failed to

trigger the process of lipid body biogenesis [15,69]; and (iii) lipid body numbers are drastically increased in blood leukocytes from septic patients or rats with Chagas disease when compared to blood leukocytes from healthy subjects [3,99], indicating that leukocytes that did not undergo migration can also form new lipid bodies *in vivo* during a systemic inflammatory disease. The lack of correlation between phagocytosis and lipid body formation was also experimentally demonstrated. By comparisons of percentages of infected cells and of cells with increased lipid body numbers after BCG infection, it was demonstrated that more than 95% of cells had highly increased lipid body numbers, while less than 30% of the cells were infected. In addition, macrophage uptake of latex beads, *Bacillus subtilis*, and nonpathogenic mycobacteria *M. smegmatis* was not able to trigger lipid body formation [15]. Together, these findings indicate that phagocytosis is neither sufficient nor essential for pathogen-induced lipid body formation, and may suggest that transfer of bacterial lipids to non- infected bystander cells and/or cytokines and other inflammatory factors generated at infection foci might contribute to lipid body formations in leukocytes. Accordingly, components from bacterial cell walls, including *E. coli* LPS and *M. bovis* BCG- or *M. tuberculosis*-derived lipoarabinomannan (LAM) can mimic the pathogen and induce lipid body accumulation in a time- and dose-dependent manner [3,15,58]. Pattern recognition receptors play major roles in regulating lipid body biogenesis during infection [100]. LPS-induced lipid body formation in macrophages was demonstrated to occur through a mechanism largely dependent on TLR-4 in cooperation with CD14 and CD11b/CD18 [3]. Both BCG and the purified cell component, LAM, failed to induce lipid body formation in macrophages and other leukocytes from TLR2-deficient mice, although lipid body formation was not modified in TLR4 deficient animals, suggesting an important role for TLR2 in this phenomenon [15,77]. Similarly, *Chlamydia pneumonia*, a pathogen that has been implicated in human and murine macrophage foam cell formation, a hallmark of early atherosclerosis, express a variety of ligands that could serve as potential TLR ligands and chlamydial infection induced macrophage lipid body formation in the presence of LDL was shown to occur through TLR2-, but not TLR4-, dependent mechanisms [101]. Interestingly, stimulation of macrophages *in vitro* with zymosan, a potent TLR2 activating agent, failed to induce lipid body formation, thus suggesting that although TLR2 activation is essential for mycobacteria-induced lipid body formation, it is not sufficient to trigger pathways of lipid body formation [15] and may involve TLR2 co-factors.

The biogenesis of lipid bodies in leukocytes is a rapidly regulated phenomenon therefore, raising the question whether lipid body formation depends on new protein synthesis. Of note, although maximum PAF-induced lipid body formation occurs within 1 hour, pretreatment of leukocytes with protein synthesis inhibitors partially inhibited PAF-, but not eotaxin- and RANTES-induced lipid body formation, thus indicating that, depending on the stimulus, induction of lipid bodies requires protein synthesis and it is likely that the transcription of early response genes is activated [3,13,39,56,70]. A role for Peroxisome Proliferator-Activated Receptors (PPARs), members of the nuclear receptor gene family that function in ligand-activated transcription, on macrophage differentiation on lipid accumulating cells has been demonstrated [102,103]. Indeed, specific PPAR γ ligands, significantly potentiate lipid body formation induced by ox-LDL, PAF-like agonists and G-CSF, suggesting that PPARs have a role in regulating leukocyte lipid body formation [73,104]. Accordingly, PPAR are capable of modulating ADRP gene transcription in different cells including macrophages [105,106].

Interestingly, leptin-induced lipid body accumulation in macrophages *in vivo* or *in vitro* is accompanied by increased levels of ADRP [93], demonstrating that leptin directly regulates the increase in ADRP cell content and accumulation of lipids within ADRP-enriched lipid bodies in macrophages and may have a role in foam cell formation. Increased ADRP expression by itself has been shown to be directly related to the enhanced capacity of neutral lipid storage, as ADRP promotes triglycerides and cholesterol storage and reduces cholesterol efflux [107]. ADRP may act also as a nucleation center for the assembly of lipids to form nascent lipid bodies

and to enhance droplet stability upon lipolytic conditions [108,109]. New findings are starting to unveil an important role for translational control by mTOR in the biogenic mechanisms of lipid bodies. We have recently established that mTOR activity is an important intracellular player in the regulation of macrophage lipid metabolism and inflammatory mediator production induced by leptin [93]. Leptin-induced ADRP-enriched lipid bodies were drastically reduced by the treatment with the mTOR inhibitor rapamycin. Taken together with the ability of leptin to induce the time- and dose-dependent P70S6K and 4EBP1 phosphorylation in a rapamycin-sensitive way, these data strongly suggest that leptin-induced increased cellular levels of ADRP depend on translational control through mTOR-dependent activation [93,94] (Figure 3). Similarly, mTOR-dependent translational control of ADRP expression has also been observed in adipocytes stimulated with conjugated linoleic acid [110]. Of note, mTOR pathway integrates signals from nutrients, energy status and growth factors to regulate many processes, including cell growth, proliferation, autophagy and metabolism [111]. Indeed, rapamycin treatment is a potent inducer of autophagy. The interplay between lipid bodies and autophagy are starting to be investigated [112,113]. Those studies suggest that lipid bodies are in close contact and act in concert with the proteasomal and autophagic pathways of protein degradation [112,113]. Intriguingly, starvation-induced autophagy in Balb-c mouse macrophages is accompanied by increases in lipid body numbers and facilitation of *Leishmania* parasite growth [114].

3.0 Lipid Body Functions in Inflammation

The compartmentalization of signaling components within discrete and dynamic sites in the cell is critical for specificity and efficiency of enzymatic reactions of phosphorylation, enzyme activation and function [115]. Spatio-temporal regulation of the different enzymes involved in cell signaling is an area of increasingly interest. Accumulating evidence has placed lipid bodies as key organelles involved in the regulation of cell signaling in inflammatory cells [69,116]. Over the past years substantial progresses have been made demonstrating that enzymes involved in eicosanoid syntheses localize at lipid bodies and lipid bodies are major sites for eicosanoid generation. Moreover, other inflammatory-relevant functions for lipid bodies have been hypothesized based on their composition and will be also discussed below.

3.1 Lipid bodies are specialized locales of eicosanoid synthesis

Eicosanoids are a family of arachidonic acid-derived signaling lipids that control important cellular processes, including cell proliferation, apoptosis, metabolism and migration [117, 118]. Thus, eicosanoids have key roles in physiological and pathological conditions such as tissue homeostasis, inflammation and cancer [117,118]. Analyses of lipid bodies in different cell types and different stimulatory conditions have demonstrated that lipid bodies are particularly active sites for the metabolism of arachidonyl lipids. Electron microscopic autoradiographic observations demonstrated that exogenous radiolabelled arachidonate was incorporated prominently in lipid bodies of eosinophils, neutrophils, mast cells, macrophages and epithelial cells [46,119–122]. Lipid bodies obtained by subcellular fractionation provided direct evidence that these organelles are stores of esterified arachidonate. In eosinophils, arachidonate was incorporated predominantly in the phospholipid pool [120]; whereas arachidonic acid-containing neutral lipids appear to be the major store of arachidonic acid in monocyte/macrophages [5,26]. Free arachidonic acid is an extremely reactive molecule that functions in cell signaling acting as an intracellular second messenger, as a paracrine mediator of cell activation and as a substrate for enzymatic conversion into eicosanoids [117,123]. Although, negligible amounts of free AA were identified in lipid bodies, different enzymes involved in arachidonic acid metabolism as well proteins involved in arachidonic acid transport were characterized in lipid bodies, thus providing strong evidence for a major role for lipid bodies in arachidonic acid metabolism. If lipid bodies are indeed involved in arachidonic acid

signaling and eicosanoid mediator formation, then the arachidonic acid present in these lipid-rich organelles must be released by phospholipases; and the free arachidonate must have access to eicosanoid-forming enzymes. cPLA₂ specifically hydrolyzes arachidonic acid from the sn-2 position of glycerophospholipids and thus serves as the rate-limiting enzyme in the formation of eicosanoids and platelet-activating factor [124]. cPLA₂ and its activating protein kinases, ERK1 and ERK2, was demonstrated to co-localize at lipid bodies [26]. cPLA₂α localizes to lipid bodies in cells responding to a wide range of stimuli, including arachidonic acid [125], Moreira, submitted). Moreover, high cPLA₂ specific activity was present in the lipid body fraction detected by measuring the release of radiolabeled arachidonic acid from the sn-2 position of 1-palmitoyl-2-[¹⁴C] arachidonyl phosphatidylcholine [26]. A large proportion of leukocyte arachidonate is stored in triglyceride pools in lipid bodies [5,26,126]. However the function and utilization of this arachidonic acid triglyceride pool has been elusive. The identification of triglyceride lipase and its activator CGI-58 within lipid bodies [29–31] opens the perspective that arachidonate from triglycerides within lipid bodies could be used as a storage pool to replenish, upon transfer, lipid body arachidonyl-phospholipids, from which regulated activation of cPLA₂ would provide arachidonate for local eicosanoid synthesis.

Proteins potentially involved in arachidonic acid transport were also shown to localize within lipid bodies. S100A9, a protein involved in the transport of arachidonate and in the shuttle of unsaturated fatty acids to membranes [127,128], was recently identified in the proteomic analysis of leukocyte lipid bodies [31]. S100A9 might participate in arachidonate-derived eicosanoid formation within leukocyte lipid bodies. Lipid bodies in neutrophils have also been shown to rapidly move and interact with phagosomes, potentially delivering lipid body-derived arachidonate to activate phagosomal NADPH oxidase [129,130].

Intracellular compartmentalization of eicosanoid synthesis within leukocytes has emerged as a key feature that regulates the amount and may also regulate the eicosanoid produced. In support of this, significant correlations between lipid body formation and enhanced generation of both 5-LO- and COX-derived eicosanoids (LTC₄, LTB₄ and PGE₂) were observed, thus indicating that lipid body numbers in leukocytes would result in enhanced capacity of eicosanoid production by leukocytes (Table 2). Indeed, stimuli known to prime leukocytes to induce eicosanoid generation, including PKC activators, arachidonate and PAF, are also active in stimulating lipid body formation [3,13,39,70,71]. Accordingly, others and we observed a significant correlation between lipid body formation and enhanced generation of both LO- and COX-derived eicosanoids *in vitro* [13,39,40,58,70,75,131] as well as *in vivo* [3,8,73,88,93, 99].

To support a role for lipid bodies in AA metabolism the location of key eicosanoid-forming enzymes were investigated by using a variety of techniques, cells and stimulatory conditions. The major enzymes, 5-LO, 15-LO, FLAP and COX, involved in the enzymatic conversion of AA into eicosanoids were shown by immunocytochemistry/immunofluorescence, ultrastructural postembedding immunogold EM and/or western blotting from subcellular fractions to localize within lipid bodies stimulated *in vitro* [38–40,54,57] or obtained from *in vivo* inflammatory responses [3,8,15,93] (Figure 4). Moreover, even the down-stream membrane spanning enzymes involved in LTC₄ production – LTC₄ synthase –, and PGE₂ production –PGE₂ synthase– have been found at lipid bodies [39,42,57].

Overall, lipid bodies compartmentalize the substrate and the entire enzymatic machinery for eicosanoid synthesis. It was recently established that successful eicosanoid production is not merely determined by AA and eicosanoid-forming enzymes availability, but requires sequential interactions between over 6 specific biosynthetic proteins acting in cascade, and may involve very unique spatial interactions. Therefore, just by detecting eicosanoid-forming enzymes within lipid bodies one cannot assure that these organelles are indeed accountable for

the efficient and enhanced eicosanoid synthesis observed during inflammatory responses. That lipid bodies can properly arrange enzymatic complexes with successful eicosanoid-forming properties and, therefore, function as specialized domains for focal eicosanoid generation has been documented by the direct intracellular localizations of newly formed eicosanoids. Direct assessment of specific intracellular sites of eicosanoid synthesis has been elusive, as those lipid mediators are newly formed, not stored and often rapidly released upon cell stimulation. By means of a strategy to covalently cross-link, capture and localize newly formed eicosanoids at their sites of synthesis, we demonstrated that lipid bodies are major intracellular locales for the activation-elicited formation of LTC₄ in eosinophils [8,56,132], LTB₄ in neutrophils and macrophages [58] and PGE₂ in macrophages and epithelial cells [57,122,133] (Figure 4).

Importantly, eicosanoid formation within lipid bodies is not restricted to leukocytes or to inflammatory conditions. Cells that produce high quantities of eicosanoids under physiological conditions, including granulosa cells of periovulatory follicles involved in the production of PGE₂ which is necessary for normal ovulation [134], luteal steroid-producing and interstitial cells involved in regression of the corpus luteum [43], and fetal membranes with advancing gestation and labor [42,135], were demonstrated to exhibit high numbers of lipid bodies containing eicosanoid synthesizing enzymes. Moreover, endothelial and epithelial cells involved in pathological conditions such as in cancer, hypoxia and during infections were shown to contain increased numbers of eicosanoid-synthesizing lipid bodies [38,54,55,57,122,136].

The roles of lipid body-derived eicosanoids may vary depending on leukocyte type, stimulus and inflammatory conditions controlling the eicosanoid synthesis. Eicosanoids synthesized at lipid body sites may function as intracellular and extracellular mediators. For instance, LTC₄ synthesized at lipid bodies within eosinophils following *in vitro* eotaxin stimulation were shown to have a novel function as an intracrine mediator regulating IL-4 secretion from eosinophils [137]. On the other hand, LTC₄ synthesized within eosinophil lipid bodies during *in vivo* allergic inflammation has paracrine activities, inasmuch as LTC₄ was released from eosinophils and was able to activate other cells present in the inflammatory site [8,9].

3.2 Storage of Cytokines

Another group of inflammatory mediators that were identified to localize within lipid bodies are cytokines — a family of glycoproteins with diverse biological activities involved in cell growth, inflammation, immunity, differentiation and repair. Cytokines and chemokines are produced and secreted by a variety of activated leukocytes including macrophages, neutrophils, mast cells, basophils and eosinophils. In addition to their ability to synthesize new cytokine proteins, leukocytes also contain cytokines as stored, preformed pools that can be found within a variety of cellular compartments, including granules, vesicles and lipid bodies [138]. Tumor necrosis factor- α (TNF- α) was the first cytokine found within lipid bodies, detected throughout cytoplasmic lipid bodies cores by immune gold EM of different cell types present in colonic Crohn's disease biopsies, including infiltrating neutrophils, macrophages and eosinophils [139]. TNF- α was also found at lipid bodies of circulating monocytes and neutrophils of septic patients and murine macrophages and neutrophils of experimentally induced sepsis [3]. Cytoplasmic lipid bodies in isolated human lung mast cells contain the basic fibroblast growth factor (bFGF), which also is present in the mast cell secretory granules [140]. Close associations of bFGF-containing lipid bodies and smooth ER, ribosomes and nuclei were observed in human lung mast cells, which may suggest a non-classical synthetic/secretory- storage pathway for bFGF in human mast cells [140].

In human eosinophils, TNF- α [139] and the lymphocyte chemoattractants RANTES and IL-16 [141] have been detected at lipid bodies. Notably, suggestive interactions between lipid bodies

and secretory vesicles involved in cytokine release in these cells termed eosinophil sombrero vesicles (EoSVs) [142,143], are frequently identified in the cytoplasm and raises a potential role for lipid bodies in vesicular trafficking (Melo, Dvorak and Weller, unpublished data; [69].

If lipid bodies represent additional subcellular storage compartments for cytokines within leukocytes, routes for cytokine-mediated secretion may exist and still need to be characterized. Alternatively, lipid body-stored cytokines may function as intracrine signaling mediators. In leukocytes, stimulus-coupled release of cytokine containing granule and/or vesicles to the cell surface for release is largely dependent on fusion mediated by the SNAREs that are present on granules/secretory vesicles and on the plasma membrane [144]. The internal membranes and cytoplasmic domains within lipid bodies may have candidate roles in the processes of vesicular transport, membrane fusion and protein secretion. Interestingly, a number of proteins likely involved in vesicular trafficking were shown to localize at lipid bodies in macrophages, including VAT-1 (synaptic vesicle membrane protein) homologue, SNAP29 (vesicle membrane fusion protein), transmembrane traffic protein, GTP binding protein SAR 1a, and Rap-1a [31]. The presence of members of the vesicular trafficking system within lipid bodies was recently confirmed by Bostrom et al, who showed the presence of NSF (*N*-ethylmaleimide-sensitive factor), α -SNAP (soluble NSF attachment protein) and the SNAREs (SNAP receptors), SNAP23 (synaptosomal-associated protein of 23 kDa), syntaxin-5 and VAMP4 (vesicle-associated membrane protein 4) at lipid bodies and demonstrated that SNARE proteins have roles in lipid body fusion processes in the cell [37]. Future studies will be necessary to characterize the regulation and function of cytokines within lipid bodies.

3.3 Compartmentalization of cell signaling

The presence of a variety of kinases within leukocyte lipid bodies has implicated this organelle as a cytoplasmic domain with compartmentalizing roles in intracellular signaling. The MAP kinases (also known as extracellular signal-regulated kinases – ERKs), as well as PKC and PI3K are key enzymes implicated in intracellular signaling of diverse cellular responses that can be found within leukocyte lipid bodies [26,44,45]. MAP kinase ERK1, ERK2 and p38 are key enzymes in the activation of cPLA₂, the enzyme that specifically hydrolyzes arachidonic acid from the *sn*-2 position of glycerophospholipids. Yu et al. demonstrated the co-compartmentalization of several MAP kinases and cPLA₂ at arachidonate enriched-lipid bodies [26]. The substantial association of cPLA₂ activators ERK1 and ERK2 with lipid bodies may serve to ensure rapid phosphorylation of cPLA₂ on lipid bodies in response to extracellular stimuli.

PI3K regulatory and catalytic subunits were also localized to lipid bodies by immunocytochemistry and/or immunoblotting and enzyme assays of subcellular fractions of isolated lipid bodies from monocyte/macrophage and polymorphonuclear leukocytes [44]. In addition, co-immunoprecipitation studies demonstrated PI3K to be physically associated with phosphorylated Lyn kinase in lipid bodies induced to form in human polymorphonuclear leukocytes [44]. Although functional studies still need to be carried out to characterize the actual roles of lipid body-resident kinases, accumulating evidence indicate that kinase-mediated signaling is active within cytoplasmic lipid bodies in leukocytes.

Novel findings are starting to reveal functions of leukocyte lipid bodies as sites of ribosomal translation and de novo protein synthesis with potential implications for the regulation of inflammation-related proteins. EM analyses of leukocyte lipid bodies demonstrated the presence of ribosomes or particles resembling ribosomal subunits. Moreover, ribosomes or ribosome subunit-like particles were present within the lipid-rich cores and/or attached to LB borders of LBs in monocyte cell line U937 and in human neutrophils and eosinophils [31].

That lipid bodies may be sites of ribosomal function is supported by the demonstration of ^3H -uridine accumulation in lipid bodies and poly (A) mRNA detection in lipid bodies by *in situ* hybridization [62,63]. Moreover, proteomic analyses of purified lipid body fractions of U937 identified several ribosomal subunit proteins as well as translation initiation factors in isolated lipid bodies [31]. Likewise, proteomic analyses of lipid bodies from hepatitis C virus core protein expressing hepatoma cell have identified ribosomal and RNA-interacting (DEAD box) proteins [66]. Does ribosomal localization within lipid bodies translate into compartmentalized protein synthesis at lipid bodies? Are there specific transcripts that are regulated within lipid bodies? Future investigations are necessary to characterize the roles of lipid bodies in the regulation of local protein synthesis during inflammation.

4.0 Involvement of lipid bodies in inflammatory disorders and potential as targets for therapeutic intervention

Lipid bodies may function as specialized intracellular sites of signaling within leukocytes engaged in inflammatory process ranging from allergy, to infections, to cancer and atherosclerosis. Inducible mechanisms that enhance eicosanoid production are attractive targets for anti-inflammatory pharmacological intervention. Although no specific lipid body inhibitor has been described so far, different classes of drugs as well as gene knockdown of PAT proteins have been demonstrated to inhibit lipid body formation. The hypothesis of lipid body inhibition as target for anti-inflammatory therapy has been tested in different model systems.

Aspirin and selected other non-steroidal anti-inflammatory drugs (NSAIDs) inhibited lipid body formation *in vivo* and *in vitro* [13,145,146]. This inhibition occurs through COX-independent mechanisms, because *cis*-unsaturated fatty acid-induced formation of new lipid bodies in macrophages from COX-1- and COX-2-deficient mice was not impaired; and NSAIDs, including aspirin, sodium salicylate, indomethacin, and NS-398, inhibited lipid body formation equally in macrophages from wild-type and COX-1- or COX-2-deficient mice [13, 145]. Pertinent to understanding the anti-inflammatory activities of aspirin, aspirin and NSAIDs, by a COX-independent mechanism, inhibited the early induction by *cis*-fatty acids of both lipid body formation and priming for enhanced eicosanoid formation in leukocytes, including notably inhibiting enhanced 5-LO pathway-mediated leukotriene generation [13]. These findings extend the anti-inflammatory capacities of aspirin and NSAIDs to include suppression of more than COX pathway-derived eicosanoids. On the other hand, in more complex stimulatory conditions, such as allergic inflammatory reactions, both COX independent and dependent processes appear to intermediate activation of lipid body-regulated lipoxygenase pathway [9].

Lipoxygenase pathway-derived *cys*-LTs (LTC_4 , LTD_4 , LTE_4) have key roles in the pathogenesis of allergic inflammatory diseases, such as asthma [147]. In fact, pharmacological blockage of *cys*-LTs synthesis/effects has been proved to be beneficial in controlling aspects of allergic pulmonary inflammation [148–150]. Therefore, one can hypothesize that the inhibitory impact on the biogenesis or function of allergen-driven lipid bodies - the *cys*LTs-synthesizing compartments *in vivo* [8,9] - may have additional therapeutic effects.

Approaches to inhibit lipid accumulation in macrophage foam cells may be of therapeutic value in preventing atherosclerosis and has been recently reviewed elsewhere [78]. Different strategies to inhibit lipid body formation have been tested to address the role of macrophage lipid bodies as targets for therapeutic intervention in atherosclerosis. ADRP expression facilitates foam cell formation induced by modified lipoproteins in mouse macrophages *in vitro*, conversely ADRP gene inactivation in apolipoprotein E-deficient mice reduces the number of lipid bodies in foam cells in atherosclerotic lesions and protects the mice against

atherosclerosis [151]. ACAT inhibitors including the fungal-derived cyclodepsipeptides showed potent inhibitory activity of lipid body accumulation in mouse peritoneal macrophages and exerted antiatherogenic activity in both low-density lipoprotein receptor- and apolipoprotein E-knockout mice [152].

The enhanced capacity of macrophages to generate PGE₂ in the course of mycobacterial infection due to increased lipid body formation and compartmentalization of stimulated local eicosanoid production within lipid bodies may contribute to the mechanisms that intracellular pathogens have evolved to survive in host cells and suggest inhibition of lipid body function as a target for pharmacological intervention in intracellular pathogen infection. Indeed, PGE₂ inhibits the Th1 type response and TNF- α and NO production [153,154]. In order to characterize the roles of NSAID-induced inhibition of formation of lipid bodies and PGE₂ on mycobacterial host response, the levels of one pro-inflammatory (TNF- α) and one anti-inflammatory cytokine (IL-10) were investigated. Accordingly, treatment with aspirin or NS 398 lead to an enhancement of TNF- α production and a drastic reduction in IL-10 generation induced by BCG-infection, that paralleled the inhibitory effect of these NSAIDs on PGE₂ and lipid body formation [15]. Similar immunomodulation of mycobacterial infection was obtained with lipid body inhibition by C75, a fatty acid synthase inhibitor [155]. Interestingly, although C75 is not a COX inhibitor, it significantly inhibited mycobacterial induced lipid body-derived PGE₂ and enhanced TNF- α production. These data suggest that lipid body induction and lipid body-derived PGE₂ down-modulate the macrophage response by inhibiting BCG-induced TNF- α production (key cytokine in mediating macrophage-induced mycobacterial killing) and increasing the levels of the anti-inflammatory cytokine IL-10 which may favor intracellular pathogen growth. Thus, inhibition of lipid body formation may be of value as a co-treatment in intracellular pathogen infections.

A role for lipid bodies as a potential target to generate new drugs for cancer treatment has been recently suggested [57]. Colonic adenocarcinoma cells contain increased numbers of lipid bodies with documented PGE₂ synthase localization and focal PGE₂ synthesis.. Inhibition of lipid body formation by either aspirin or a fatty acid synthase inhibitor correlated with both inhibition of PGE₂ generation and cell proliferation in CACO-2 and IEC-6 H-rasV12 cells [57]. The inhibition of lipid body generation may affect the subcellular compartmentalization of COX-2 and in consequence inhibit the enhanced prostaglandin synthesis that is related to the pathogenesis of colon cancer.

Future studies will be necessary to characterize the role of lipid bodies as targets for therapeutic intervention in diseases that progress with increased lipid body accumulation as in atherosclerosis, hepatic steatosis, cancer and inflammation. That to include further safety characterization of lipid body inhibition as lipid accumulation within lipid bodies may act as protective mechanisms in lipid homeostasis against cellular lipotoxicity. Moreover, the development of selective lipid body inhibitors is in need.

6.0 Concluding Remarks

Major advances in the understanding of the cellular and molecular mechanisms regulating leukocyte lipid body biogenesis and functions were achieved in recent years. Our contemporary view of lipid bodies places this organelle as critical regulators of different inflammatory and infectious diseases and key markers of leukocyte activation. Notably, leukocyte lipid body biogenesis is highly regulated and is cell and stimuli specific. Studies of lipid body structural features have revealed a much more complex structure than initially anticipated that beside lipids includes a diverse array of proteins that may vary according to the cell type and cellular activation state and thus may determine different cellular functions for lipid bodies. Moreover, internal membranes and ribosomes were identified within leukocyte lipid bodies adding to the

system complexity. In leukocytes and other cells, it have been established that lipid bodies are specialized, inducible cytoplasmic domains that have central roles to control the synthesis and secretion of inflammatory mediators. However, for an organelle centrally involved in cellular lipid balance and cellular signaling, our current understanding of its biogenesis, dynamics, heterogeneity and function in different cellular systems are still very limited. Critical open questions remain about the formation and functions of lipid bodies not only to understand normal leukocyte function but also in several inflammatory-related diseases. In conclusion, recent studies place lipid bodies as multifunctional organelles with key function in lipid storage and cell signaling in inflammation and as such are emerging as attractive target candidate for therapeutic intervention.

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Abbreviations

5-LO	5- lipoxygenase
AA	arachidonic acid
ACAT	acyl-coenzyme A cholesterol acyltransferase
ADRP	adipose differentiation related protein
BCG	Bacillus Calmett Guérin
COX	cyclooxygenase
cPLA₂	cytosolic phospholipase A ₂
EDAC	1 – ethyl – 3 (3 – dimethylamino – propyl) carbodiimide
ER	endoplasmic reticulum
EM	electron microscopy
FLAP	5- lipoxygenase activating protein
IL	interleukin
LDL	low density lipoprotein

LPS	lipopolysaccharide
LT	leukotriene
MAP	mitogen-activated protein
MCP-1	monocyte chemotactic protein-1
NSAID	non-steroidal anti-inflammatory drug
PI3K	phosphatidylinositide 3 kinase
PG	prostaglandin
PKC	protein kinase C
PPAR	Peroxisome Proliferator-Activated Receptor
TIP 47	tail-interacting protein of 47 kDa
TLR	toll-like receptor

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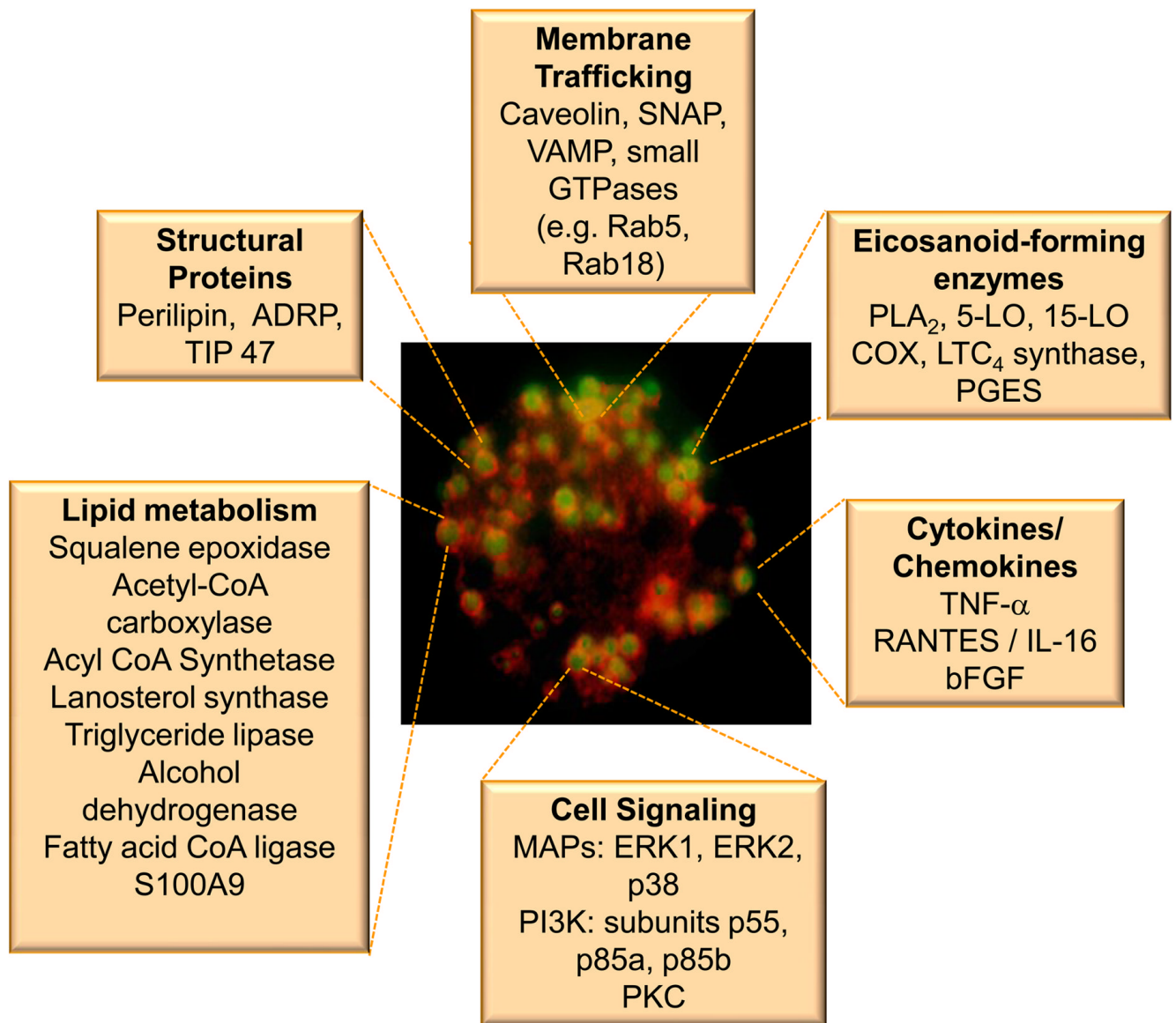


Figure 1.
Lipid body-associated proteins

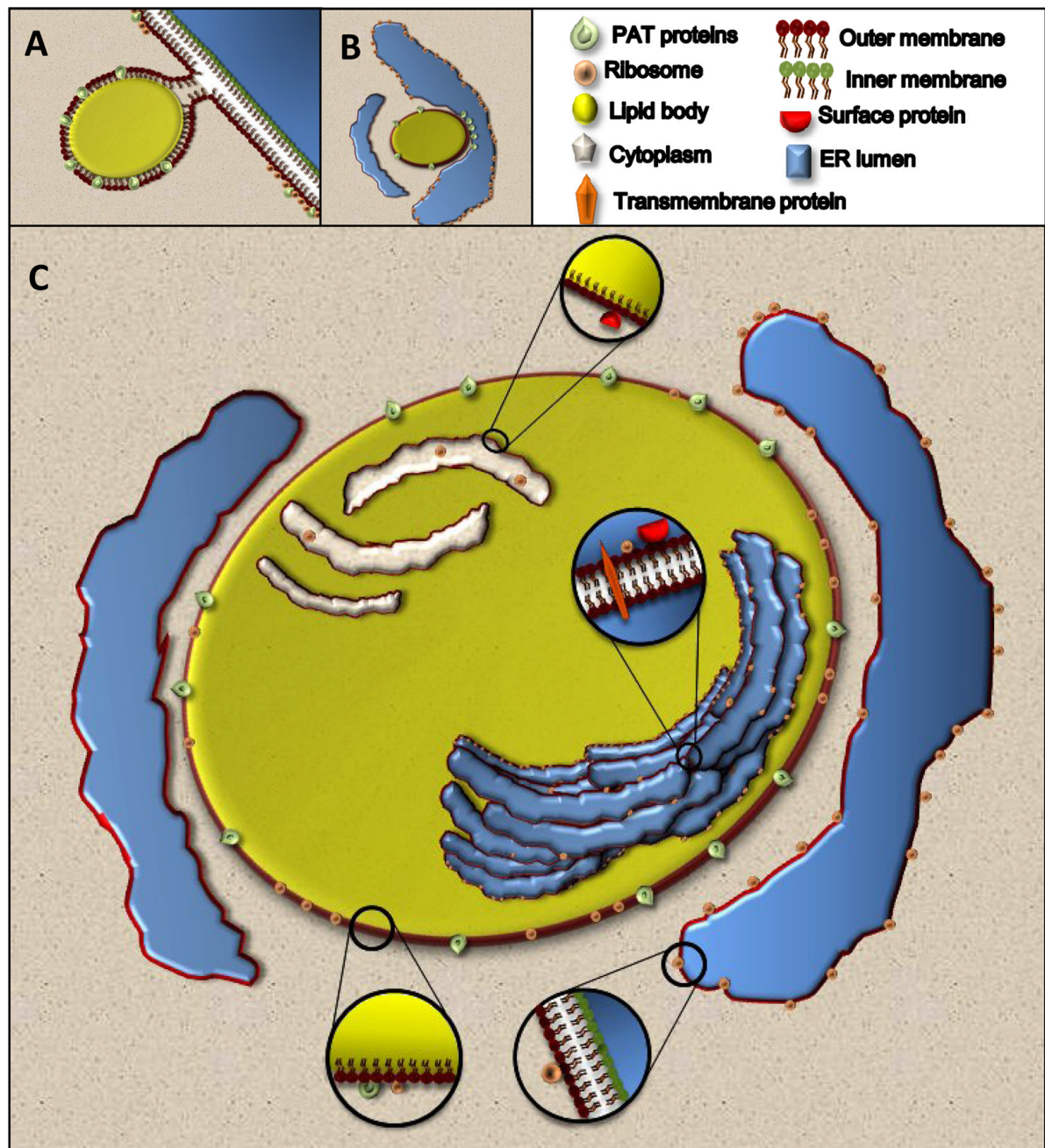


Figure 2. Biogenesis and structure of lipid bodies

Lipid body biogenesis involves ER-dependent mechanisms of lipid and protein transference. Three main models have been proposed: (A) formation of a neutral lipid mass synthesized by ER enzymes that is deposited in a hydrophobic domain between the two leaflets of the ER membrane; followed by budding off of this lipid structure into the cytoplasm that ends up surrounded by a half-unit membrane of phospholipids directly derived from the cytoplasmic leaflet of the ER; (B) formation of lipid bodies at ADRP-enriched clusters in the cytoplasmic leaflet of ER with the transference of lipids from ER to nascent lipid bodies within ER cups, rather than between ER leaflets; (C) formation of lipid bodies by incorporation of multiple loops of ER membranous domains, with accumulation of neutral lipids among the membranes

within lipid bodies. As detailed in C, ultrastructural and proteomic studies of lipid bodies isolated from leukocytes revealed internal membranes within these leukocyte lipid bodies and identified diverse proteins, about half of which had predicted membrane insertions [31]. In addition, ribosome and ribosomal-associated proteins have also been identified within lipid bodies.

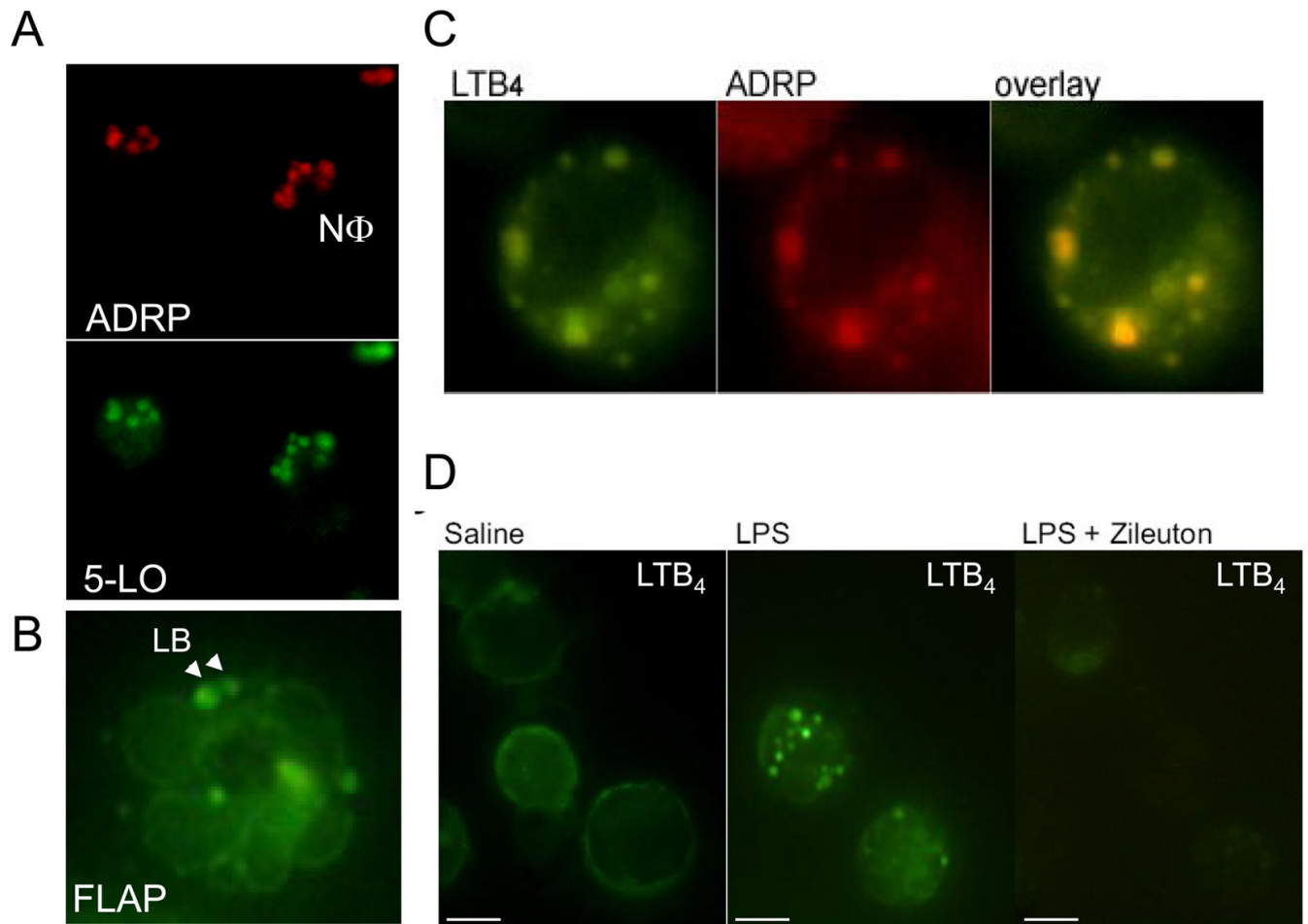


Figure 3. Schematic representation of the molecular mechanisms regulating leukocyte lipid body biogenesis and function

Lipid body formation in leukocytes is a highly regulated process that involves receptor-mediated signaling. Lipid body formed during inflammatory stimulation culminates in the compartmentalization of lipids and proteins and function as specialized domains involved in eicosanoid synthesis.

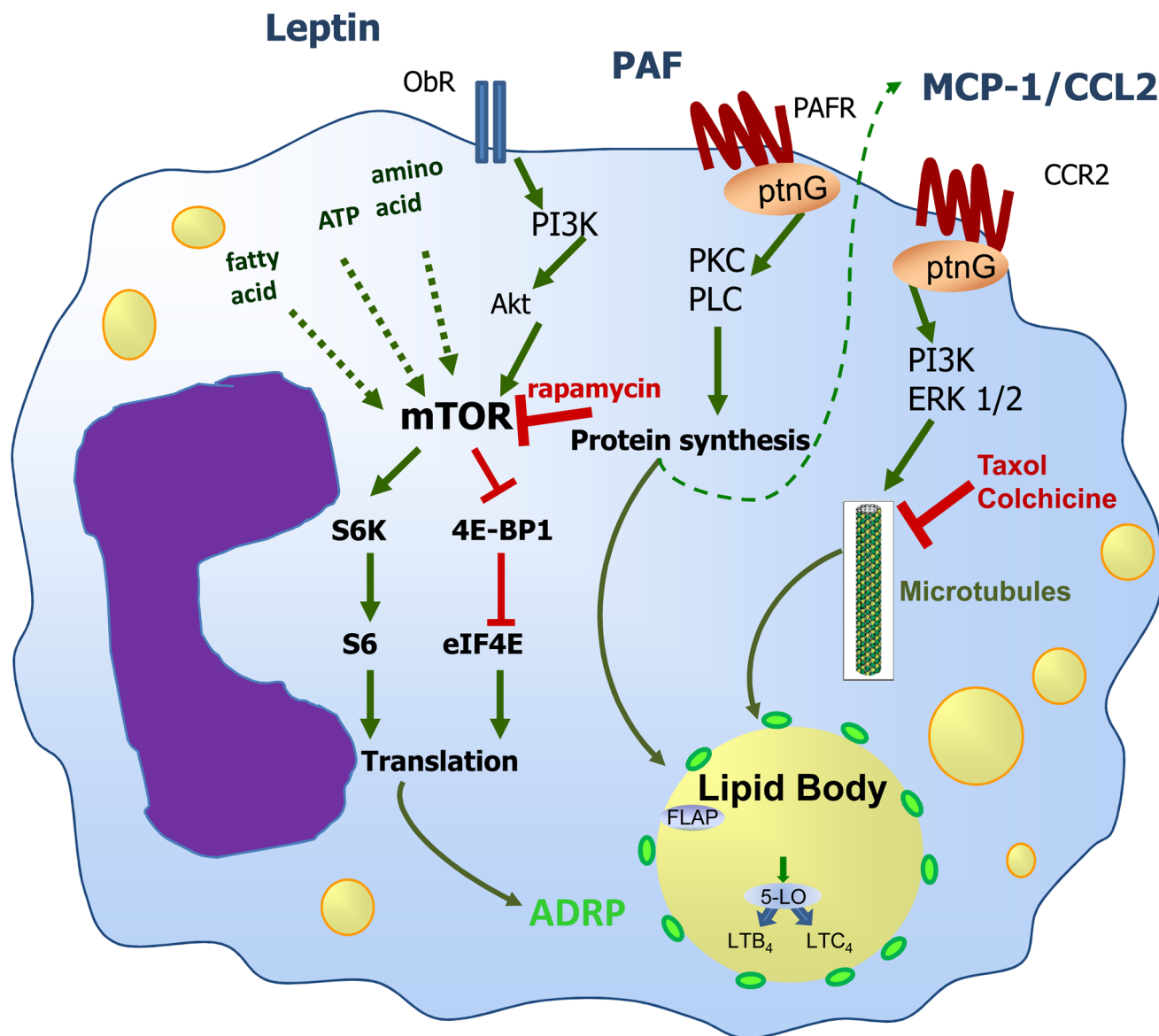


Figure 4. Leukocyte lipid bodies are intracellular locales for eicosanoid synthesis in inflammation

Leukotriene forming enzymes 5-LO (A) and FLAP (B) are localized at leukocyte lipid bodies obtained from in vivo LPS-stimulated animals. In A, colocalization of 5-LO at ADRP-labeled In C and D, Newly formed eicosanoids were immobilized at its site of synthesis by cross-linking of the lipids to adjacent proteins using 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDAC). LTB₄ were identified by using anti-LTB₄ and revealed with Cy2-labeled anti-rabbit IgG. In C, Images show LTB₄ immuno-reactive lipid bodies (as identified by anti-ADRP) of peritoneal macrophages. In D, Leukocytes from in vivo vehicle- (right panel) or LPS-stimulated animals (middle panel) were immunolabeled for newly formed LTB₄ after LPS stimulation. LPS-stimulated animals were treated with the 5-LO inhibitor zileuton before incubation with EDAC (left panel), demonstrating that eicosanoid production at lipid bodies is a regulated and enzymatic-dependent process. Images show that LTB₄ immunolabeling has a punctate cytoplasmic pattern that is absent in nonstimulated cells,

inducible by LPS, and sensitive to zileuton (Part C and D were reproduced with permission from [58] Copyright 2007 The American Association of Immunologists, Inc.).

Table 1
Leukocyte lipid body formation is cell- and stimuli-dependent

Stimuli	Cell Type	References
Fatty Acids		
Unsaturated fatty acids, but not saturated fatty acids	Neutrophils, eosinophils, monocyte/macrophages	13,71,131
Lipoproteins		
Ac-LDL/E-LDL/AGE-LDL/Ox-LDL, but not native LDL	Macrophages, but not neutrophils	1,2,73,79–82,102
Pathogen-derived molecules		
LPS/LAM	Macrophages, neutrophils	3,15
Lipid mediators		
PAF, but not lysoPAF	Neutrophils, eosinophils, macrophages	39,40,70,73
5-HETE	Neutrophils	70
PGD ₂	Eosinophils but not macrophages	9
Hormones		
Leptin, resistin	Macrophages	92,93
Cytokines/Growth factors		
IL-5, GM-CSF, IL-16	Eosinophils	40,75,76
Chemokines		
CCL5/CCL11/CCL24/CCL26	Eosinophils	56,74
CCL2	Macrophages	58

Table 2
Increased leukocyte lipid body formation is involved in priming for eicosanoid production

Cell	Stimulus	LTC ₄	LTB ₄	PGE ₂	Ref
Human PMNs	Oleic acid		+	+	13,131
	Arachidonic acid		+	+	13,131,145
	Platelet Activating Factor (PAF)		+	+	70,131
	Phorbol ester (PMA)		+		131
Human Monocytes	Lipopolysaccharide (LPS)		+	+	3
Human Eosinophils	Oleic acid	+			13
	Arachidonic acid	+			13
	Oleyl-acetyl-glycerol (OAG)	+			131
	PAF	+		+	39,40
	Eotaxin/CCL11	+			56,74
	CCL24/CCL26	+			74
	RANTES/CCL5	+			59
	IL-5-IL-5R (via PAF)	+			40,75
	IgG-FcγR (via PAF)	+			75
	PGD ₂	+			9
Human Eosinophil cytoplasts	IL-16 (via CCL11/CCL5)	+			74
	PAF	+		+	39
Mouse macrophages	Arachidonic acid			+	13
	Leptin		+	+	93
	CCL2		+		58
Mouse PMN + macrophages	LPS		+	+	3,58
	PAF and PAF-like		+	+	70,73